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EVALUATION REPORT

Pantoea agglomerans strain E325

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FOREWORD

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, has granted conditional registration for the sale and use of the technical grade active ingredient Bloomtime Biological Technical Biopesticide containing the microbial pest control agent *Pantoea agglomerans* strain E325 and the end-use product, Bloomtime Biological FD Biopesticide for suppression of *Erwinia amylovora*, the causative agent of fire blight disease in apple and pear orchards. These products were reviewed jointly as biopesticide products within the North American Free Trade Agreement's Technical Working Group on Pesticides (NAFTA TWG) Joint Review Program by the PMRA and the United States Environmental Protection Agency (USEPA).

Pantoea agglomerans strain E325 in Bloomtime Biological FD Biopesticide is a biological antagonist that suppresses *E. amylovora* by competing against it for space and resources on apple and pear trees. The E325 strain of this species is naturally occurring and has not been genetically modified.

Microbial pest control agents are increasingly investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment. Bloomtime Biological FD Biopesticide represents a potential biological replacement for chemical pesticides.

Current scientific data from the registrant and published scientific reports were evaluated to determine if, under the proposed conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Evaluation Report summarizes the information that was evaluated, provides the results of the evaluation, describes the conditions that are required to ensure that the health and environmental risks and the value of these pest control products are acceptable for their intended use and provides the reasons for the conditional registration decision (with an outline of the additional confirmatory scientific information requested).

As these conditional registrations relate to a decision on which the public must be consulted[1], a public consultation document on the proposed decision will be published at the time that a decision is required to either convert these conditional registrations to full registrations or to continue them as conditional registrations.

The information in this Evaluation Report is presented in two parts. The Overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessment of Bloomtime Biological FD Biopesticide.

Also included is a List of References that indicates both the studies and information submitted by the registrant as well as the additional information considered by the Agency in support of this registration decision.

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[1] As per subsection 28(1) of the *Pest Control Products Act* 2002.

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OVERVIEW

Registration Decision for Bloomtime Biological FD Biopesticide

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, has granted conditional registration for the sale and use of Bloomtime Biological Technical Biopesticide containing the microbial pest control agent *Pantoea agglomerans* strain E325 and the end-use product Bloomtime Biological FD Biopesticide for suppression of *Erwinia amylovora*, the causative agent of fire blight disease in apple and pear orchards.

Current scientific data from the registrant and published scientific reports were evaluated to determine if, under the proposed conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Evaluation Report summarizes the information that was evaluated, provides the results of the evaluation, describes the conditions which are required to ensure that the health and environmental risks as well as the value of the pest control products are acceptable for their intended use and provides the reasons for the conditional registration decision (with an outline of the additional confirmatory scientific information requested).

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its conditions or proposed conditions of registration.[2] The Act also requires that products have value[3] when used according to label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

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2 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*

3 "Value"as defined by subsection 2(1) of the *Pest Control Products Act*: "...the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

To reach its decisions, The PMRA applies hazard and risk assessment methods as well as policies that are rigorous and modern. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g., children) as well as organisms in the environment (e.g., those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties present when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.pmra-arla.gc.ca.

What is Bloomtime Biological FD Biopesticide?

Bloomtime Biological FD Biopesticide is a biological pesticide containing the bacteria *P. agglomerans* strain E325. The product is applied to blossoms of apple and pear trees in commercial apple and pear orchards. *Pantoea agglomerans* strain E325 is a naturally occurring bacteria of fruit trees. Once on fruit blossoms, it quickly multiplies on the flowers to outcompete and displace other bacteria, including *E. amylovora*, a destructive bacteria that causes the fire blight disease. The growth of *P. agglomerans* strain E325 on fruit blossoms simply suppresses the ability of *E. amylovora* to grow and reach levels necessary to trigger fire blight disease development.

❖ Health Considerations

♦ Can Approved Uses of Bloomtime Biological FD Biopesticide Affect Human Health?

***Pantoea agglomerans* strain E325 is unlikely to affect your health when Bloomtime Biological FD Biopesticide is used according to label directions.**

Exposure to *P. agglomerans* strain E325 may occur during handling and applying of the product. When assessing health risks, the following key factors are considered:

- the microorganism's biological properties (e.g., production of toxic byproducts);
- reports of any adverse incidents;
- its potential for causing disease, infection and toxicity, as determined in toxicological studies; and
- the likely levels to which people may be exposed relative to exposures already encountered in nature to other strains of the microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses, which may help us identify any concerns related to potential disease, infection and toxicity. When *P. agglomerans* strain E325 was tested on laboratory animals, no significant toxicity and no signs of a disease-causing source or infection were observed.

Other strains of *P. agglomerans* found in nature have been associated with minor wound infections involving punctured skin; however, there is no indication that infection would arise if healthy skin is penetrated. This species of microorganism does not cause disease in humans and is not known to produce byproducts that are harmful to humans or other animals.

Pantoea agglomerans strains produce a substance on their cell walls called lipopolysaccharide that can be shed from the cells as tiny "pockets". If inhaled in large amounts, the lipopolysaccharide of *P. agglomerans* can cause inflammation in the respiratory system. Upon repeated exposure to this product, a condition known as respiratory hypersensitivity could therefore develop in people such as farm workers and applicators. Like all bacteria, *P. agglomerans* strain E325 contains other substances that can cause allergic reactions in people who are repeatedly exposed to it at high concentrations. However, these reactions can be avoided if farm workers and applicators follow label recommendations to minimize or limit exposure to Bloomtime Biological FD Biopesticide.

♦ **Residues in Water and Food**

Dietary risks from food and water are not of concern.

Pantoea agglomerans strains are common in nature, and application of Bloomtime Biological FD Biopesticide to apple and pear trees is not expected to significantly increase the natural environmental background levels of this microorganism. Few bacteria are expected to remain as residues on the fruit at harvest because the product is applied to fruit trees at bloom time well before fruit are present. No adverse effects have been attributed to dietary exposure from natural populations of *P. agglomerans*. When *P. agglomerans* strain E325 was administered orally to rats, there was no significant toxicity, and no signs of a disease-causing source was observed. Furthermore, there are no reports of known toxins to mammals being produced by the bacteria. Therefore, the establishment of a maximum residue limit is not required for *P. agglomerans* strain E325.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* Each MRL value defines the maximum concentration in parts per million of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk. Furthermore, the likelihood of residues of *P. agglomerans* strain E325 contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risk are minimal to non-existent.

♦ Occupational Risks From Handling Bloomtime Biological FD Biopesticide

Occupational risks are not of concern when Bloomtime Biological FD Biopesticide is used according to label directions, which include protective measures.

Pesticide applicators handling or applying Bloomtime Biological FD Biopesticide and field workers re-entering orchards where trees were sprayed can come into direct contact with *P. agglomerans* strain E325 on the skin, in the eyes or by inhalation. For this reason, the label will specify that farm workers exposed to Bloomtime Biological FD Biopesticide must wear waterproof gloves, a long-sleeved shirt, long pants, shoes, socks and a dust/mist filtering mask. Furthermore, early-entry workers will be restricted from entering orchards treated with Bloomtime Biological FD Biopesticide for up to four hours after spraying unless they are wearing the appropriate personal protective equipment.

For bystanders, exposure is expected to be much less than that of field workers and is considered negligible. Therefore, health risks to bystanders are not of concern.

❖ Environmental Considerations

♦ What Happens When Bloomtime Biological FD Biopesticide is Introduced Into the Environment?

Environmental risks are not of concern.

There are no published reports of disease associated with *P. agglomerans* in wild mammals, birds, earthworms, bees and other arthropods, aquatic invertebrates, fish, algae and aquatic plants. Therefore, Bloomtime Biological FD Biopesticide is expected to present a negligible risk to these non-target organisms. Only rare cases of disease caused by wild strains of *P. agglomerans* have been reported in plants, including cotton, onion, garlic and beach pea as well as in seedlings of such conifer (evergreen) species as Douglas fir. *Pantoea agglomerans* does not affect apple or other pome fruit trees. Given the narrow range of plant species that have been infected by wild strains of this bacteria and the limited use pattern of strain E325 in apple and pear orchards, the likelihood of non-target plants of commercial or environmental importance being affected by Bloomtime Biological FD Biopesticide is minimal. However, as a measure to protect commercially important stands of conifer trees, the product label will instruct users to avoid spraying orchards adjacent to newly planted conifer forestry blocks.

❖ Value Considerations

♦ **What is the value of Bloomtime Biological FD Biopesticide?**

Bloomtime Biological FD Biopesticide, a biological pesticide, suppresses the fire blight disease in apple and pear orchards.

To provide effective suppression of fire blight in orchards, Bloomtime Biological FD Biopesticide should be applied to apple and pear trees twice per season. The first application should be made at early bloom (15–20% in bloom stage), and the second application at full bloom or once petals have fallen. Bloomtime Biological FD Biopesticide is compatible with streptomycin and should be used in an integrated fire blight suppression program with streptomycin. Copper-based formulations are not compatible with Bloomtime Biological FD Biopesticide.

Measures to Minimize Risk

Registered pesticide product labels include specific instructions for use. Directions include risk reduction measures to protect human and environmental health. These directions are required by law to be followed.

The key risk-reduction measures on the label of Bloomtime Biological FD Biopesticide to address the potential risks are as follows:

Key Risk-Reduction Measures

- **Human Health**

Because of a concern with users developing allergic reactions through repeated high exposures to *P. agglomerans* strain E325, anyone handling or applying Bloomtime Biological FD Biopesticide must wear waterproof gloves, a long-sleeved shirt, long pants, shoes plus socks and a dust/mist filtering mask. Furthermore, early-entry workers will be restricted from entering orchards treated with Bloomtime Biological FD Biopesticide for up to four hours after spraying unless the appropriate personal protective equipment is worn.

- **Environment**

As wild strains of *P. agglomerans* have been known to cause gall disease in some conifer tree species, including the commercially important Douglas fir, the Bloomtime Biological FD Biopesticide label will direct users to avoid applying the product to apple or pear orchards that are adjacent to newly planted conifer forestry blocks.

What Additional Scientific Information Is Being Requested?

Although the risks and value have been determined to be acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional confirmatory scientific information is being requested from the registrant as a result of this evaluation (see below) to ensure that *P. agglomerans* strain E325 can be properly distinguished from other strains of *P. agglomerans* and to confirm the absence of any human and animal disease-causing sources from the final formulated product. For more details, refer to the Section 12 Notice associated with these conditional registrations. The registrant will be asked to submit this information within the time frames indicated below.

- **Methods**

  The following items are required to permit identification of the microbial pest control agent and to confirm the absence of disease-causing sources in the final formulated product.

  - An identification method to distinguish strain E325 from other naturally occurring strains of *P. agglomerans* is required. A genetic fingerprinting method has been developed for other *P. agglomerans* strains (McManus and Jones 1995), which could readily be adapted for this purpose. Submission of the method to the Agency must be made no later than 1 December 2007.

  - To ensure that Bloomtime Biological FD Biopesticide does not contain any human and animal disease-causing "microorganisms", the registrant will be required to include microbe-specific screening methods for *Salmonella*, *Shigella*, *Staphylococcus*, enteric bacteria, *Vibrio*, yeasts and moulds as well as *Pseudomonas aeruginosa* in the manufacturing process. Confirmatory data from five representative production batches will be required. Alternatively, if fewer than 5 batches of end-use product are manufactured in a 12-month period, then representative data from each batch produced in that timeframe will be acceptable. Submission of the method and representative data to the PMRA must be made no later than 1 December 2007.

Other Information

As these conditional registrations relate to a decision on which the public must be consulted[4], a public consultation document on the proposed decision will be published at the time that a decision is required to either convert these conditional registrations to full registrations or to continue them as conditional registrations.

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[4] As per subsection 28(1) of the *Pest Control Products Act*.

The test data cited in this Evaluation Report (i.e., the test data relevant in supporting the registration decision) will be available for public inspection after the public consultation process on the proposed decision has been completed and a decision is made. If more information is required, please contact the Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra_infoserv@hc-sc.gc.ca).

SCIENCE EVALUATION

Pantoea agglomerans strain E325

1.0 The Active Substance, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Pantoea agglomerans* strain E325
Function	Suppress *Erwinia amylovora* populations (fire blight) on apple and pear trees
Binomial name	*Pantoea agglomerans* strain E325
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Pantoea*
Species	*agglomerans*
Strain	E325
Patent status information	United States patent number: 5919446
Nominal purity of active ingredient	1×10^{10} CFU/g
Identity of relevant impurities of toxicological, environmental and/or significance	The technical grade active ingredient does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards and, except for lipopolysaccharide (a component of all Gram-negative bacteria), no mammalian toxins are known to be produced by *Pantoea agglomerans* strain E325.

1.2 Physical and Chemical Properties of the Active Substances and End-Use Product

Technical Product—Bloomtime Biological Technical Biopesticide

Property	Result
Colour	Not applicable
Odour	Not applicable
Physical state	Not applicable
Formulation type	Not applicable
Guarantee	Not applicable
Container material and description	Not applicable
Density	Not applicable
pH of 1% dispersion in water	Not applicable
Oxidizing or reducing action	Not applicable
Storage stability	Not applicable
Explodability	Not applicable

End-Use Product—Bloomtime Biological FD Biopesticide

Property	Result
Colour	Light yellow
Odour	Not reported
Physical state	Powder
Formulation type	Live organism (LO)
Guarantee	336 g/L (limits: 325–345 g/L)
Container material and description	Aluminum foil-lined pouches
Density	0.195 g/mL
pH of 1% dispersion in water	Not reported
Oxidizing or reducing action	Not applicable
Storage stability	27 months at between -10°C and 4°C
Explodability	Not applicable

1.3 Details of Uses and Further Information

Bloomtime Biological FD Biopesticide is a powder-formulated end-use product containing 7.0% w/w *Pantoea agglomerans* strain E325 (minimum guarantee of 1×10^{10} CFU/g as the sole active ingredient. It is proposed to suppress fire blight (*Erwinia amylovora*) disease on apples and pears.

Bloomtime Biological FD Biopesticide is proposed for use at 375 grams product per hectare, to be applied at the 15–20% bloom stage, followed by a second application at full bloom to petal fall stages in 500–1500 L of water per hectare. For a more diluted spray, use 500 grams of product per hectare in 2000–3000 L of water per hectare. Ensure thorough coverage of blooms.

Bloomtime Biological FD Biopesticide is compatible with streptomycin. Copper-based formulations are incompatible with Bloomtime Biological FD Biopesticide. The microbial pest control agent (MPCA) was selected by the United States Department of Agriculture (USDA), Agricultural Research Service researchers as a spontaneous rifampicin-resistant isolate. The isolate was then selected for streptomycin resistance on antibiotic-amended plates.

1.4 Mode of Action

The primary mode of action appears to be competitive exclusion. Following application on trees, *P. agglomerans* strain E325 is able to reproduce on the blooms for several days, effectively colonizing the tree and occupying sites otherwise colonized by the fire blight pathogen (*E. amylovora*).

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Methods to identify uniquely the MPCA are a key component of manufacturing quality assurance. The MPCA was identified to species based on colony and cell morphological characteristics using conventional bacteriological media, gas chromatography of fatty acid methyl ester (GC-FAME), carbon utilization method for bacterial identification (Biolog), and sequence analysis of the 16S rDNA gene.

GC-FAME and Biolog identify isolates by biochemical profiles as compared with a library of species' profiles, based on an "average" of hundreds of isolates of each species. A good match (i.e., a positive identification) is indicated by a similarity coefficient greater than 0.5. The best match for GC-FAME and Biolog analysis was with *P. agglomerans*, with similarity indices of 0.805 and 0.759, respectively.

Sequence analysis of the 16S rDNA gene was carried out using primers M13-reverse or T7 primers followed by amplification. Another strain also developed for biological control of fire blight, *P. agglomerans* strain C9-1 (Ishimaru et al. 1988), was also sequenced and aligned with *P. agglomerans* strain E325. Results showed that the two strains were different by only four nucleotides. Although the 16S rDNA sequencing data demonstrated a four nucleotide difference

between the E325 and C9-1 strains, the method has not been used to distinguish the MPCA strain from other commonly encountered strains of *P. agglomerans*.

There was no method submitted for strain-specific identification. The registrant will be required to develop a method using the best available technology to distinguish the E325 strain from other naturally occurring isolates of *P. agglomerans*.

2.2 Methods for Establishment of Purity of Seed Stock

The original source of *P. agglomerans* strain E325 is stored and maintained at -70°C at the USDA laboratory in Wenatchee, Washington. Working stocks are also maintained at the USDA laboratory as a lyophilized preparation at -20°C, and on silica gel at -20°C, for convenient retrieval. The cultures are replenished periodically by sprinkling silica gel stock onto agar media. No further details on replenishing the stocks was provided.

Production cultures are prepared by the USDA in 4% milk suspended onto silica gel, stored at -4°C in sealed glass vials, and shipped frozen overnight to a manufacturing facility in Pasco, Washington, on an as required basis. Upon arrival, the vials are dated and assigned a lot number and stored frozen at -4°C.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/mL) of the product is routinely checked throughout the manufacturing process by plating collected samples on antibiotic-amended nutrient-yeast-dextrose-agar (NYDA) plates. Stored manufactured product (fermentation broth) is analyzed for potency by the same method prior to the final formulation step (i.e., freeze-drying) to ensure that all fermentation broths used for additional processing meet the product guarantee. Finally, the freeze-dried end-use product is also checked for potency (CFU/g) following the same method to ensure the product meets the label guarantee of 1×10^{10} CFU/g prior to distribution.

2.4 Methods to Determine and Quantify Residues (viable or non-viable) of the Active Microorganism and Relevant Metabolites

Although *P. agglomerans* is ubiquitous in nature and has been isolated from a wide variety of environments, no adverse effects from dietary exposure have been attributed to natural populations of *P. agglomerans*. In a 14-day oral toxicity/pathogenicity study in which Bloomtime Biological FD Biopesticide was delivered to Sprague-Dawley rats by gavage in a single oral dose of 1.05×10^8 CFU/animal, the MCPA was detected in the cecum contents, brain, kidney, lungs and spleen of treated rats on day 7, but was completely cleared from all organs and fluids by day 14. There were no signs of toxicity or pathogenicity observed and all animals appeared healthy. Although the MPCA clearance data from interim sacrifices were equivocal, the MPCA was shown to be non-toxic and non-pathogenic when administered orally.

Adverse effects attributable to bacterial lipopolysaccharide are not expected on oral exposure, and there are no reports of other mammalian toxins being produced by the MPCA. Furthermore,

the pesticide is not expected to come into direct contact with the fruit because of the timing of its application (during bloom). The establishment of a maximum residue limit (MRL) is therefore not required for *P. agglomerans* strain E325 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drug Regulations.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

Microbial contamination during the manufacturing of Bloomtime Biological FD Biopesticide is minimized by sterilizing the starting and intermediate materials as well as the equipment.

Serial dilutions of samples of the product (e.g., fermentation broth) are screened for microbiological contamination on standard NYDA plates and are visually examined for contamination. Stored manufactured product is also screened for contaminants by the same method prior to the final formulation step (i.e., freeze-drying) to ensure that all fermentation broths used for additional processing are absent for contaminating organisms. A final screening for contaminants on standard NYDA plates is also carried out on freeze-dried end-use product prior to distribution.

The presence of any contaminant on NYDA plates results in termination of the batch; however, this plating method is inadequate for detecting and enumerating human and animal microorganisms of concern (see the following Section).

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

Plating fermentation broth sampled at various time points in the manufacturing process on non-selective NYDA is inadequate for detecting and enumerating microbiological contaminants of concern. No methods were submitted to screen specifically for human and mammalian pathogens during the manufacture of Bloomtime Biological FD Biopesticide. Microbe-specific methods to screen for human and animal pathogens such as *Salmonella*, *Shigella*, *Staphylococcus*, enteric bacteria, *Vibrio*, yeasts and moulds as well as *Pseudomonas aeruginosa* must be included in the manufacturer's quality assurance program.

2.7 Methods to Determine Storage Stability, Shelf-Life of the Microorganism

Storage stability data were collected from two batches of end-use product stored for approximately 15 days and from one batch stored for 8, 10, 12 and 27 months. The storage temperature was not specified in the report, but the product was presumably stored between -10°C to 4°C (frozen or refrigerated), as specified on the proposed product label. Based on the limited data provided from one batch, the end-use product appears to be stable for up to 27 months under these conditions.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *P. agglomerans* strain E325. The database is largely complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity and acute pulmonary toxicity/pathogenicity) currently required for health hazard assessment purposes that were carried out in accordance with currently accepted international testing protocols and good laboratory practices. A waiver request was deemed acceptable to address the dermal toxicity, intraperitoneal toxicity/pathogenicity, primary eye irritation and dermal irritation in lieu of testing. The scientific quality of the data is high, and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

A survey of the published literature indicated that, under favourable conditions, *P. agglomerans* (formerly known as either *Erwinia herbicola* or *Enterobacter agglomerans*) can act as an opportunistic pathogen, causing systemic infection in parenterally exposed patients, especially those with predisposing factors such as immunosuppression, a history of antibiotic use, prolonged hospital stays, the presence of invasive devices such as venous catheters and premature birth or low birth weight in neonates (Sanders and Sanders 1997). Because the synonym *E. agglomerans* is the generally accepted nomenclature in clinical literature, it will be used in the following discussion. Unambiguous identification of *E. agglomerans* in clinical isolates is challenging. In case reports, when the causative agent was identified to species, this was done using cell or colony morphology, cultural and biochemical characteristics (in later reports, using commercially available identification kits such as API 20E, ID-32E and Biolog). Genetic methods were rarely used to confirm identification of putative *E. agglomerans* isolates.

Localized, suppurative *Enterobacter* infections are common at the site of puncture wounds, usually from woody materials. Arthritis is often noted near the site of the wound (von Graevenitz and Strouse 1966, von Graevenitz 1971, Gilardi et al. 1970, Pien et al. 1972, Mason et al. 1976, Flatauer and Khan 1978, Olengienski et al. 1991, De Champs et al. 2000, Durr et al. 2001, Kratz et al. 2004, Uiloa-Gutierrez et al. 2004). Each of these cases was successfully treated with a series of antibiotics.

Cases of postoperative *Enterobacter* septicemia, including septicemia caused by *E. agglomerans*, were also reported (Mildvan et al. 1971, Meyers et al. 1972, Pien et al. 1972). *Enterobacter* respiratory infections were also prominent. In some cases, patients had no underlying illness to predispose them to infection (Pien et al. 1972).

Enterobacter agglomerans has been implicated in outbreaks of nosocomial infection related to contaminated intravenous products, including anaesthetics, parenteral nutrition and blood (Meyers et al. 1972, Felsby et al. 1973, Maki et al. 1976, Matsaniotis et al. 1984, Bennett et al. 1995, Goncalves et al. 2000, Hasbah et al. 2005). In some outbreaks, organisms such as *Enterobacter cloacae*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Serratia* species were also isolated from the contaminated products, and it was not clear which organism was responsible for mortalities. Mortalities were most common in neonates and those with predisposing factors such as immunosuppression, but this did not appear to be a requirement for infection.

Eye infections have also been reported. Mirza et al. (1994) described an outbreak of postoperative *Enterobacter* endophthalmitis in six patients following a single day of surgery, which was traced to unsterilized cotton swabs. Although the contaminating organism was not identified to species, *E. agglomerans* is a known colonizer of cotton (Rylander and Ludholm 1978). Only one of the seven affected eyes retained useful vision after a follow-up period of two years. A case of acute conjunctivitis in a 70-year old (Mason et al. 1976) after a puncture wound with a thorny brier branch had long-term sequelae in spite of antibiotic treatment. Bacteria cultured from fluid in the aqueous chamber of the eye was identified by morphological, cultural and biochemical characteristics as a species of the *Erwinia herbicola-lathyri* group, also designated as *E. agglomerans*.

Given the ubiquitous nature of the species, however, infection with *P. agglomerans* is rare. Cases identified in the literature were opportunistic in nature, and the healthy human population is not expected to be at greater risk from exposure to Bloomtime Biological FD Biopesticide than from exposure to native populations of *P. agglomerans*. It is important that a relatively rapid method be developed to permit the unique identification of *P. agglomerans* strain E325 from commonly encountered strains from nature. A genetic fingerprinting method has been developed for other *P. agglomerans* strains (McManus and Jones 1995), which could readily be adapted for this purpose.

An acute oral toxicity/pathogenicity study and an acute pulmonary toxicity/pathogenicity study with *P. agglomerans* strain E325 were submitted. In the oral toxicity/pathogenicity study, the MCPA was detected in the cecum contents, brain, kidney, lungs and spleen of treated rats on day 7, but was completely cleared from all organs and fluids by day 14. In the pulmonary toxicity/pathogenicity study, the MPCA was detected in the kidneys of treated rats from day 3 to day 14, but completely cleared from all animals by day 21. In both studies, there were no signs of toxicity or pathogenicity, and all animals appeared healthy at all observation times. Although the MPCA clearance data in interim sacrifices were equivocal, the MPCA was shown to be non-toxic and non-pathogenic when administered orally and by intratracheal instillation.

A waiver request for the outstanding health studies (dermal toxicity/pathogenicity study, an intraperitoneal toxicity/pathogenicity study, a primary eye irritation study and a dermal irritation study) based on a comprehensive review of published literature was found to be acceptable to fully assess the risks associated with the MPCA given the intended use pattern of the end-use product in lieu of toxicity testing.

As a Gram-negative bacterium, the cell wall of *P. agglomerans* strain E325 contains lipopolysaccharide (LPS). LPS rapidly activates the innate immune response, characterized by the production of the inflammatory mediators interleukin-1 and tumour necrosis factor alpha (de Rochemonteix-Galve et al. 1991, Kuby 1994). Symptoms of respiratory exposure may include dry cough, shortness of breath, decrease in lung function, fever, malaise, dyspnea, headache and joint aches (Heederik and Douwes 1997). Although LPS is characteristic of all Gram-negative bacteria, a review of the published literature indicates that *P. agglomerans* LPS is exceptionally potent (Tsukioka et al. 1997). The endotoxic properties of *E. herbicola* LPS were demonstrated by Dutkiewicz (1976) who showed that it was lethal in mice (LD_{50} 0.23–0.50 mg), produced primary inflammatory lesions in rabbit skin and prepared rabbit skin for the local Schwartzman reaction (i.e., hemorrhagic necrosis developed at the site of primary skin lesions following intravenous injection of endotoxin). In guinea pigs, inhalation of aerosol preparations of lyophilized endotoxin of *P. agglomerans* increased breathing rate and resulted in a significant pulmonary influx of inflammatory cells and changes in the ultrastructure of alveolar macrophages (Milanowski 1994b).

In humans, occupational diseases, including organic dust respiratory syndrome in grain and herb farmers, as well as byssinosis in cotton workers, are thought to be primarily due to exposure to LPS. *Pantoea agglomerans* has been specifically implicated in these diseases (Dutkiewicz 1997, Wang et al. 2005). *Enterobacter agglomerans* LPS is also associated with "cotton-fever" (Ferguson et al. 1993), an acute, febrile reaction with chest tightness and bronchoconstriction. These symptoms arise in drug users, after intravenous injection of drugs when they are using cotton as a filter.

Dutkiewicz et al. (1992) demonstrated that *E. herbicola* sheds LPS-containing membrane microvesicles, which have been shown to elicit strong inflammatory responses in rabbit upon repeated exposure by inhalation (Dutkiewicz et al. 2005). Such microvesicles may be more readily inhaled than the organism itself, possibly explaining the association of *P. agglomerans* with occupational respiratory syndromes. To mitigate the risk of respiratory exposure to *P. agglomerans* strain E325 LPS during and postapplication of Bloomtime Biological FD Biopesticide, personal protective equipment and a restricted-entry interval will be required.

Although no incidents of hypersensitivity were reported during development of Bloomtime Biological FD Biopesticide, *P. agglomerans* is a known sensitizing component of agricultural dusts. Numerous hypersensitivity reactions in grain and herb workers have also been reported, as indicated by a higher incidence of positive skin prick and precipitin reactions to *P. agglomerans* soluble antigen in grain workers as compared with rural inhabitants or urban-dwelling controls (Dutkiewicz 1978b, Dutkiewicz et al. 1985, Milanowski et al. 1998, Dutkiewicz et al. 2001, Spiewak et al.2001, Golec et al. 2004, Golec 2006). Exposure to allergens, including *P. agglomerans* allergens, in organic dust has been related to many occupational diseases such as asthma, allergic alveolitis, allergic rhinitis, airborne contact dermatitis, conjunctivitis and systemic allergic reactions (Dutkiewicz 1997, Golec 2006). All microbial pesticides are considered to be potential sensitizers. Label statements indicating that the Bloomtime Biological FD Biopesticide is a potential sensitizer and label precautions requiring personal protective equipment and judicious handling to minimize exposure in workers will be required.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and because there were no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *P. agglomerans* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *P. agglomerans* strain E325.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of applicator exposure to *P. agglomerans* strain E325.

The potential for dermal, eye and inhalation exposure for mixer/loaders, applicators and early-entry workers exists, with the major source of exposure to workers being dermal. Because unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were broken, if the microbe were a pathogen equipped with mechanisms for entry through the skin or if metabolites were produced that could be dermally absorbed. *Pantoea agglomerans* has been identified as a wound pathogen, causing local infections at the site of puncture wounds, but there is no indication that it could penetrate intact skin. It is not known as a human pathogen, though cases of opportunistic infection have been reported in the literature. It is not known to produce metabolites that are dermally absorbed. Based on the published literature, it is unlikely that a systemic infection could result from the penetration of the dermal barrier in an otherwise healthy individual.

The risk of a respiratory inflammatory reaction to LPS (endotoxin) exists in individuals exposed by inhalation to the MCPA, and based on cases in the published literature, respiratory hypersensitivity could be expected to develop upon repeated exposure to the product. Specific label wording to minimize spray drift should minimize exposure of bystanders to airborne spray mists. Exposure in mixer/loaders, applicators and early-entry workers will be mitigated by a restricted-entry interval and a label requirement for personal protective equipment, including a particulate filter mask.

Although no dermal toxicity or irritation studies were submitted for the MPCA, all MPCAs are considered potential sensitizers. Label restrictions and risk-mitigation measures are required to protect populations that are likely to be primarily exposed to the pesticide. Such exposure to mixer/loaders, applicators and early-entry workers can be minimized if they wear gloves, a long-sleeved shirt, long pants, shoes and socks. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. In addition, several reports in the published literature suggest that *P. agglomerans* is a sensitizing agent. The signal words

"POTENTIAL SENSITIZER" will be required on the principal display panel of the technical grade active ingredient and end-use product labels.

Although Bloomtime Biological FD Biopesticide contains other ingredients (formulants), these are not expected to be irritating to the eyes. Published literature on *P. agglomerans* indicated a potential for pulmonary inflammation, and the submitted acute pulmonary toxicity/pathogenicity study suggests that the MPCA may remain in the lungs for up to 14 days prior to complete clearance. However, inhalation exposure is not a concern if the required dust/mist filtering respirator is worn by mixer/loaders, applicators and early-entry workers, preferably a MSHA/NIOSH approval number prefix TC-21C or a NIOSH-approved respirator with any –95, R-95, P-95 or HE filter for biological products, to minimize inhalation exposure. To minimize dermal, inhalation and eye exposure as well as risk to workers, use of appropriate personal protective equipment will be stipulated on the end-use product labels as will a restricted-entry interval of four hours.

3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *P. agglomerans* strain E325 and the assumption that precautionary label statements will be followed in the use of Bloomtime Biological FD Biopesticide to minimize off-target spray drift.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Bloomtime Biological FD Biopesticide is applied to pome fruit trees at the flowering stage; consequently, the proposed food use pattern is unlikely to result in significant residues on treated fruit at the time of harvest. While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children or animals because *P. agglomerans* strain E325 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Dietary exposure to secondary metabolites produced by *P. agglomerans* strain E325 is also not expected, given the proposed use pattern of Bloomtime Biological FD Biopesticide. Furthermore, higher-tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MCPA and because there were no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity studies. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Although *P. agglomerans* strain E325 could enter neighbouring aquatic environments via spray drift or surface-water runoff and can potentially survive in water, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimum and it showed no harmful effects on animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. Specific product labelling will be required to limit spray drift and surface water runoff. The potential for transfer of *P. agglomerans* strain E325 to surface water or groundwater during runoff is considered minimal to non-existent due in part to its percolation through and resulting capture in soil, where the organism can be found naturally. The Bloomtime Biological FD Biopesticide label instructs users not to allow the product to enter bodies of water during use or disposal. Furthermore, municipal treatment of drinking water will reduce the transfer of residues to drinking water. Therefore, potential exposure to *P. agglomerans* strain E325 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses and acceptable daily intakes is not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MCPAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *P. agglomerans* strain E325 is of low toxicity, is not pathogenic or infective to mammals and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intraspecies and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from prenatal or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *P. agglomerans* strain E325 to human health.

3.4 Maximum Residue Limits

Although *P. agglomerans* species are ubiquitous in nature and have been isolated from a wide variety of environments, application of Bloomtime Biological FD Biopesticide is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse effects from dietary exposure have been attributed to natural populations of *P. agglomerans*. Furthermore, no adverse effects were observed in the acute oral toxicity/pathogenicity study, and there are no reports of known mammalian toxins being produced by the MPCA. Therefore, the establishment of an MRL is not required for *P. agglomerans* strain E325 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drug Regulation. The Act

prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established MRL. Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value defines the maximum concentration in parts per million of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *P. agglomerans* strain E325 to the general Canadian population, including infants and children, when the MCPA is used according to label directions. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. The product is to be applied to outdoor agricultural sites and is not allowed for use on turf residential or recreational areas; therefore, dermal and inhalation exposure to the general public will be very low. Furthermore, no significant clinical signs were observed in laboratory animals exposed orally or by pulmonary instillation to *P. agglomerans* strain E325 at maximum hazard doses, and there is limited information on adverse effects from exposure to other strains of *P. agglomerans* encountered in the environment. Even if there is an increase in exposure to this microorganism from the use of Bloomtime Biological FD Biopesticide, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. All Gram-negative bacteria contain LPS as part of the cell wall. Although the inflammatory effect of LPS is a concern on exposure to large quantities of *P. agglomerans*, this effect is not expected to be cumulative. Besides naturally occurring strains of *P. agglomerans* in the environment and the *P. agglomerans* strain C9-1 found in the commercial biopesticide BlightBan C9-1, the Agency is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *P. agglomerans* strain E325 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *P. agglomerans* strain E325. Environmental fate data (Tier II/III) are not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental fate testing is intended to demonstrate whether an MCPA is capable of surviving or replicating in the

environment to which it is applied. These results could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent of exposure. Some information on the environmental fate of *P. agglomerans* strain E325 is available in the published literature.

Johnson et al. (2000) investigated the spread of another strain of *P. agglomerans* strain C9-1 between inoculated and non-inoculated pear and apple trees. *Pantoea agglomerans* strain C9-1 is also used for biological control of fire blight. In the experiment, bacteria were applied to the three central rows of trees in an orchard block, and blossoms were sampled from inoculated and non-inoculated trees for the presence and population density of the bacteria. Immediately after inoculation, the MCPA was detected on blossoms from inoculated trees, but not on non-inoculated trees. As bloom progressed, the size of the population on inoculated trees increased, and non-inoculated trees up to 18 metres from the nearest inoculated tree had high proportions of blossoms colonized by *P. agglomerans* strain C9-1. The colonization of inoculated blossoms with the MPCA as well as the dissipation of the organism to non-inoculated blossoms was favoured by periods of warm, dry weather and limited by periods of cooler, wet weather. The authors hypothesized that warm weather favoured colonization and dissipation because of a higher bacterial growth rate on inoculated trees and on non-inoculated trees following dissipation from the treated row. Warm, dry weather also favoured increased insect activity, and this appeared to favour dissipation, as bees were important vectors for transfer of the bacteria between blossoms of the inoculated and non-inoculated trees. *Pantoea agglomerans* strain E325 is expected to behave similarly to *P. agglomerans* strain C9-1.

The ubiquity and diversity of habitats used by *P. agglomerans* suggest that it will survive under field conditions. The organism is best understood as an epiphyte of plants. It occurs in many parts of the plant, e.g., in the phyllosphere of *Rosa rugosa* (Hashidoki et al. 2002), salad vegetables (Brocklehurst et al. 2002, Hamilton-Miller and Shah 2001) and herbs (Golec et al. 2004), on the stem of sweet potato (Asis Jr. and Adachi 2003), on buckwheat seeds (Iimura and Hosono 1996) and in the rhizosphere of oilseed rape (Berg et al. 2002). It has also been isolated from aquatic environments (as *E. agglomerans*; Brown and Leff 1996) and from recirculated water in industrial settings (Laitinen et al. 1999). As a facultative anaerobe, it has also been isolated as an iron-reducing bacterium from the anaerobic sediments of a marine coastal basin (Francis et al. 2000). Costa et al (2002) investigated the growth requirements of a biocontrol strain, *P. agglomerans* strain CPA-2, and delimited its growth range with respect to water availability (a_w 0.95–0.96), temperature (1–42°C) and pH (5–8.6). The limits on growth and reproduction of *P. agglomerans* strain E325 were not submitted.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

No studies were submitted to address the risks of Bloomtime Biological FD Biopesticide to terrestrial organisms. Therefore, the potential risk of *P. agglomerans* to terrestrial organisms was assessed based on reports in the published scientific literature.

For terrestrial vertebrates, no reports of adverse effects in wild mammals or bird populations were found in the published literature. Because *P. agglomerans* is ubiquitous in the environment, wild mammals and bird populations are considered to have been exposed to indigenous populations of the organisms, with no incidents of adverse effects reported. Furthermore, no hazards from *P. agglomerans* strain E325 for wild mammalian species are anticipated for this use. The laboratory animal studies on the rat submitted in support of this registration and reviewed in Section 3.1 indicate that there is no toxicity or pathogenicity to rodents from testing at maximum hazard dose levels. The rodent results support a waiver for testing of wild mammals as well as for birds. Additional risk to birds and wild mammals may occur from LPS contained within the cell wall of *P. agglomerans* which has been shown to elicit strong immunomodulative properties in rabbits upon repeated exposures (Dutkiewicz et al. 1992, Dutkiewicz et al. 2005). Even though LPS causes effects in humans and in rabbits by stimulating the immune system through pathways common to mammals and birds, such adverse effects in non-target animals would only be expected to occur upon exposure to large quantities of aerosolized bacterial endotoxin. This exposure is not anticipated based on the proposed use of Bloomtime Biological FD Biopesticide in orchards.

For terrestrial arthropods (including honeybees), published studies in which honeybees were directly dusted with pollen coated with *P. agglomerans* as vector delivery systems for biological control of fruit trees reported no adverse effects (Thompson et al. 1992, Vanneste 1996, Vanneste et al. 2002). In another study, ladybird beetles were fed *E. herbicola* strain 265G-2 as an ice-nucleating bacterium (Strong-Gunderson et al. 1990) with no incidents of adverse effects reported. It should be noted, however, that adverse effects were not the experimental outcome of interest in the studies, so only effects severe enough to compromise the results of the study would likely have been reported. These reports offer evidence, based on a worst-case exposure scenario (i.e., direct dusting), that exposure to the MPCA will not result in adverse effects on honeybees. Furthermore, the applicant reports that there have been no incidents of negative impact to beehives in the test plot orchards in limited field trials with Bloomtime Biological FD Biopesticide over the past three years.

Other published reports indicated that *P. agglomerans* is a common organism of the gut microbiota of the following organisms:

- mosquitoes (*Culex quinquefasciatus*, Pidiyar et al. 2004; and Anopheles funestus,; Straif et al. 1998);
- locusts (Dillon et al. 2002); and
- the apple maggot fly (*Rhagoletis pomonella*, Lauzon et al. 2003).

Pantoea agglomerans (*E. agglomerans*) was also identified in association with sheep scab mites (*Psoroptes ovis*, Hogg and Lehane 2001) and as an intracellular symbiotic bacterium of the cereal weevil (*Sitophilus oryzae*, Heddi et al. 1998).

For earthworms and other soil macroorganisms, no study was submitted to address the risks of Bloomtime Biological FD Biopesticide to earthworms or other non-arthropod invertebrates. Effects data are not required because the product is not intended to control pest non-arthropod invertebrates or soil macroorganisms and proposed use patterns do not indicate a potential for adverse effects.

For other soil microorganisms, no study was submitted to address the risks of Bloomtime Biological FD Biopesticide to soil microorganisms. Effects data are not required although the product is intended to control pest microorganisms, as *P. agglomerans* is a normal component of the soil and the organism is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes.

For terrestrial plants, published literature indicated that *P. agglomerans* is ubiquitous in the environment and is recognized as an epiphyte of a wide variety of plants, such as follows:

- buckwheat (Iimura and Hosono 1996);
- weeds (Gavini et al. 1989);
- oilseed rape (Berg et al. 2002)
- sweet potato (Asis and Adachi 2003);
- rice (Komagata et al. 1968) and
- trees of the Rosaceae family (Hashidoko et al. 2002, Nunes et al. 2001).

Pantoea agglomerans is found on a wide variety of plant parts, including the rhizosphere, leaves and seeds. The species is also a heavy colonizer of cotton plants (Rylander and Ludholm 1978), grass and silage (Heron et al. 1993) and is the prominent species in organic dust (Prażmo et al. 2003; Krysińska-Traczyk et al. 2004; Skórska et al. 2005). The organism has also been isolated from soil and water (Gibbins 1978; Gavini et al. 1989; Brown and Leff 1996). Recent reports have also identified *P. agglomerans* on retail salad vegetables (Brocklehurst et al. 1987, Hamilton-Miller and Shah 2001).

Erwinia herbicola has been implicated, though rarely, in infections of several plant species, including the following:

- cotton (Ashworth et al. 1970);
- onion (Kritzman and Zutra 1984);
- garlic (Koch et al. 1996);
- beach pea (pathogen identified as *P. agglomerans*, Khetmalas et al. 1996); and
- Douglas fir (DeYoung et al. 1998).

In the Douglas fir study, *E. herbicola,* isolated from a slow-growing, smooth-surfaced circular gall on Douglas fir, induced gall formation on stab inoculation of several conifer species (*Abies amabilis, A. grandis, A. lasiocarpa; Chamaecyparis nootkatensis; Larix occidentalis; Picea engelmannii, P. glauca, P. sitchenisis; Pinus contorta, P. monticola, P. ponderosa; Thuja plicata; Tsuga heterophylla*), all economically important softwood lumber species. Douglas fir remained the most susceptible host. The presence of these galls affected the health and structural integrity of the host tree. Death of the inoculated branch or branch tips occurred 2–4 months

after gall formation. Galls on the main stem of young Douglas fir seedlings (less than six months old) often killed the seedlings. Trees greater than one year old did not appear to be significantly affected.

Several efficacy trials have been also been conducted with Bloomtime Biological FD Biopesticide, including 11 laboratory and field trials (4 on crabapples, 6 on apples and 1 on pears) at up to 25 times the rate on the proposed product label. No phytotoxicity or phytopathogenicity was reported in any of the laboratory, greenhouse or field trials after application of Bloomtime Biological FD Biopesticide. There have also been no reports of phytotoxicity from published research conducted with other strains of *P. agglomerans* also used for fire blight suppression (Pusey 1997, Johnson et al. 2000).

Based on a review of existing scientific information and literature available on the effects of *P. agglomerans* to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates or to other microorganisms from the proposed use of Bloomtime Biological FD Biopesticide in apple and pear orchards. Therefore, the PMRA has accepted the request to waive terrestrial non-target organism testing. Limited data available on the effects of environmental isolates of *P. agglomerans* on plants suggest a potential for infection of conifer seedlings, particularly Douglas fir. Consequently, the Agency will require a precautionary statement on the product label recommending that users avoid applying product to orchards abutting newly planted conifer forestry blocks.

4.2.2 Effects on Aquatic Organisms

There were no reports of disease or adverse effects in fish or other aquatic organisms related to any *Pantoea* species in the published literature. Use of Bloomtime Biological FD Biopesticide will be limited to a foliar application in apple and pear orchards only. This intended use pattern minimizes direct exposure to non-target aquatic organisms. Although the product is not intended for direct application to water, spray drift and surface water runoff from treated orchards may result in contamination of aquatic ecosystems. Published literature indicated that several strains of *P. agglomerans* (*E. agglomerans*) have been isolated from aquatic habitats (Brown and Leff 1996), suggesting that the MCPA could survive in aquatic ecosystems. Any *P. agglomerans* strain E325 that reaches aquatic ecosystems in the form of runoff, overspray or spray drift is expected to behave as any *P. agglomerans* strain would in nature. Although the absence of reports of disease or adverse effects in literature suggests that adverse effects are unlikely, effects in aquatic organisms have not specifically been investigated and it is possible that incidents may occur. However, as noted above, there are no reports in the literature indicating toxicity or pathogenicity of *P. agglomerans* to non-target aquatic organisms including fish, invertebrates and plants; therefore, there is reasonable certainty of no harm without further testing, and the requirement for non-target testing on aquatic organisms is waived.

5.0 Value

5.1 Effectiveness Against Pests

The efficacy data package submitted in support of the claims for suppression of fire blight on apples and pears with Bloomtime Biological FD Biopesticide consisted of 11 laboratory and field trials in total (4 on crabapples, 6 on apples, and 1 on pears).

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Suppression of *Erwinia amylovora* Population

Pantoea agglomerans strain E325 (Bloomtime Biological FD Biopesticide) and two other biopesticides, BlightBan C9-1 (*P. agglomerans* strain C9-1) and BlightBan A506 *(Pseudomonas fluorescens* strain A506), were tested to determine their ability to suppress *E. amylovora* on the stigma of crabapple, pear and apple. Among the three biological control products tested, treatment with Bloomtime Biological FD Biopesticide resulted in the highest suppression (75.8–99.9%) of the fire blight pathogen *E. amylovora* (strain 153) on the stigma of greenhouse-grown crabapple as well as field-grown pears and apples. Although Bloomtime Biological FD Biopesticide gave numerically the greatest level of suppression of fire blight, the two other biopesticides tested also provided a significantly similar level of suppression of *E. amylovora* (strain 153).

In two trials, Bloomtime Biological FD Biopesticide was applied twice at 20–30% of bloom stage, and again at 70–80% bloom at the rate of 371–741 g product/ha (26.0–51.9 g a.i./ha or 1.0–1.5 times of the proposed rates) in 1000 litres of water. This resulted in 28–44% control of fire blight incidence compared to the untreated control. In two other trials, another formulation of Bloomtime Biological FD Biopesticide (with a lower guarantee) was applied at much higher rates, 1.24–9.63 kg product/ha (87.0–674.0 g a.i./ha) in 1000 L of water/ha, made at 20–30% bloom and at 70–80% bloom. This formulation provided 16.4–58% control of fire blight on apples.

In another trial, Bloomtime Biological FD Biopesticide was applied alone prior to inoculation with the fire blight pathogen at 2×10^7 CFU/mL at 20–30% bloom and at 60–80% bloom stages and in combination with Agrimycin 17 WP applied after inoculation. This combination provided 58% and 64.5% control of blossom blight on apple. Agrimycin 17 WP applied alone provided 77.5% control of blossom blight. Results indicate that Bloomtime Biological FD Biopesticide does not provide as good control of fire blight as streptomycin, but it can be used as a companion product in an integrated fire blight suppression program on pears and apples.

Complete efficacy data or scientific rationales testing all the aspects of the proposed application rates and water volumes were not submitted for review. Based on the use pattern used in different trials, the application rates of 375–500 g product/ha in 1000–2000 litres of water are supported.

5.1.1.2 Tank-Mix Combinations

No information regarding tank-mixing of Bloomtime Biological FD Biopesticide with other registered pest control products was submitted for review.

5.2 Phytotoxicity to Target Plants

5.2.1 Acceptable Claims for Host Plants

Development of phytotoxicity and phytopathogenicity was not reported in any of the laboratory, greenhouse or field trials after application of Bloomtime Biological FD Biopesticide. Therefore, it is believed that Bloomtime Biological FD Biopesticide is not phytotoxic or phytopathogenic on pears and apples.

5.3 Impact on Succeeding Crops

Data on the impact of Bloomtime Biological FD Biopesticide on succeeding crops were not submitted for review.

5.4 Economics

No market analysis was done for this submission.

5.5 Sustainability

5.5.1 Survey of Alternatives

Fire blight disease on apples and pears is currently managed by cultural practices such as removal of overwintering cankers during the dormant season and growing relatively tolerant cultivars. Few products (Streptomycin 17, Apogee plant growth regulator and various copper-based fungicides/bactericides) are registered in Canada for control or suppression of fire blight.

5.5.2 Compatibility With Current Management Practices Including Integrated Pest Management

The most common and most efficacious product for fire blight control is the antibiotic-based product Streptomycin 17. There are, however, concerns that the fire blight pathogen *E. amylovora* may develop resistance to streptomycin. Thus, there is a need for alternative products for fire blight management to reduce the use of streptomycin. Bloomtime Biological FD Biopesticide is compatible with streptomycin and can be used as a companion product in an integrated fire blight suppression program with this product. Copper-based formulations, however, are incompatible with the performance of Bloomtime Biological FD Biopesticide.

5.5.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

No information is available on the risk of development of resistance in the population of *E. amylovora* after multiple applications of Bloomtime Biological FD Biopesticide over a longer period of time.

As this strain of *P. agglomerans* was isolated from natural microflora on apple blossoms and the mode of action against *E. amylovora* appears to be competitive exclusion, the risk of *E. amylovora* strains developing resistance to Bloomtime Biological FD Biopesticide after multiple applications is very low.

5.5.4 Contribution to Risk Reduction and Sustainability

Bloomtime Biological FD Biopesticide is a microbial pest control product whose mode of action is based on competitive inhibition and exclusion of the fire blight disease-causing organism *E. amylovora* on apple and pear trees. It is the only non-chemical product intended as an alternative to streptomycin to suppress this disease. As a microbial biopesticide, the PMRA considers it to be a reduced-risk pesticide that has a low potential to harm the health of Canadians and their environment.

6.0 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances resulting predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *P. agglomerans* strain E325, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product Bloomtime Biological Technical Biopesticide and formulants in the end-use product Bloomtime Biological FD Biopesticide. The PMRA has reached the following conclusions:

- *Pantoea agglomerans* strain E325 does not meet the Track 1 criteria because the active ingredient is a biological organism; therefore, it is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

- Bloomtime Biological Technical Biopesticide does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

- The end-use product Bloomtime Biological FD Biopesticide does not contain any formulants of health or environmental concern identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

Therefore, the use of Bloomtime Biological FD Biopesticide is not expected to result in the entry of Track 1 substances into the environment.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for both *P. agglomerans* strain E325 and Bloomtime Biological FD Biopesticide are adequate to assess their safety to human health. The technical material was fully characterized, but no method was submitted to distinguish strain E325 from other naturally occurring strains of *P. agglomerans*. Development of a method will be required as a condition of registration. The molecular-based method described and shown by McManus and Jones (1995) to reliably differentiate strains of this bacterial species is suggested as a reference method. No methods were submitted to screen for human and mammalian pathogens during the manufacture of Bloomtime Biological FD Biopesticide. A method to screen for the possible presence of human and animal pathogens, such as species of *Salmonella*, *Shigella*, *Staphylococcus*, *Vibrio*, enteric bacteria, yeasts and moulds as well as *Pseudomonas aeruginosa* is required. Potency analysis of the active ingredient and microbiological contaminant screening data from five representative batches of Bloomtime Biological FD Biopesticide are also required. Alternatively, if fewer than five batches of end-use product are manufactured in the first year of registration, then representative data from each production batch of that year will suffice.

7.2 Human Health and Safety

Acute toxicity and infectivity studies (acute oral toxicity and pulmonary toxicity) and published literature submitted in support of *P. agglomerans* strain E325 and Bloomtime Biological FD Biopesticide were determined to be acceptable. *Pantoea agglomerans* strain E325 was of low toxicity and infectivity in the rat when administered via the oral and pulmonary route.

Pantoea agglomerans is a known sensitizing component of agricultural dusts with numerous reports of hypersensitivity reactions in grain and herb workers. Exposure to allergens, including *P. agglomerans* allergens, in organic dust has been related to many occupational diseases such as asthma, allergic alveolitis, allergic rhinitis, airborne contact dermatitis, conjunctivitis and systemic allergic reactions. All microbial pesticides are considered to be potential sensitizers. As a result, the signal words "POTENTIAL SENSITIZER" are required on the principal display

panels of the labels for both the technical grade active ingredient and the end-use product. In addition, as an eye irritation study was not submitted on the end-use product or the technical grade active ingredient, the signal words "CAUTION EYE IRRITANT" are required on the principal display panels of both product labels.

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of applicator and bystander exposure to *P. agglomerans* strain E325. While submitted studies on strain E325 and the published literature on *P. agglomerans* indicated a potential for pulmonary inflammation, inhalation exposure is not a concern if the required dust/mist filtering respirator is worn by mixer/loaders, applicators and early-entry workers, preferably a MSHA-NIOSH approval number prefix TC-21C or a NIOSH-approved respirator with any –95, R-95, P-95 or HE filter for biological products. To minimize dermal, inhalation and eye exposure as well as risk to workers, use of appropriate personal protective equipment will be stipulated on the end-use product labels, as will a restricted-entry interval of four hours. The label does not allow applications to turf, residential or recreational areas. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Although *P. agglomerans* species are ubiquitous in nature and have been isolated from a wide variety of environments, no adverse effects from dietary exposure have been attributed to natural populations of *P. agglomerans*. Furthermore, no adverse effects were observed in the acute oral toxicity and infectivity study and there are no reports of known mammalian toxins being produced by the MCPA. Therefore, the establishment of an MRL is not required for *P. agglomerans* strain E325 under Section 4(d) of the *Food and Drugs Act* (adulteration of food), as defined under Division 15, Section B.15.002 of the Food and Drug Regulations.

7.3 Environmental Risk

Adverse effects in mammals were assessed in studies described under Section 3.0. Adverse effects are not anticipated in wild mammals or in birds, based on a lack of toxicity or infectivity in acute oral and pulmonary studies on laboratory rats. Moreover, adverse effects are not expected in earthworms, bees and other arthropods, aquatic invertebrates, fish, algae as well as aquatic plants, as no reports of disease have been published in the scientific literature.

Pantoea agglomerans is ubiquitous in nature and naturally occurring on fruit trees. Often identified by researchers as *E. herbicola*, it has been associated with necrotic infections in terrestrial plants including, cotton, onion, garlic, beach pea, and Douglas fir; however, incidents of disease attributable to *P. agglomerans* appear to be rare. Because of the economic importance of softwood lumber, a study showing gall formation due to *E. herbicola* in Douglas fir and other Western conifer species is of some concern. Precautionary labelling advising applicators not to spray Bloomtime Biological FD Biopesticide near newly planted forestry blocks should be sufficient to prevent the inoculation and possible galling of young seedlings through wounds inflicted during planting.

Although Bloomtime Biological FD Biopesticide is not intended for direct application to water, spray drift and surface water runoff from treated orchards may result in contamination of aquatic ecosystems. Several strains of *E. agglomerans* have been isolated from aquatic habitats (Brown and Leff 1996), suggesting that *P. agglomerans* strain E325 could survive in aquatic ecosystems. Although risk to aquatic non-target organisms is expected to be minimal to non-existent, precautionary labelling is required on the end-use product label to reduce spray drift and runoff into aquatic ecosystems adjacent to treated apple and pear orchards.

7.4 Value

Application of Bloomtime Biological FD Biopesticide at a rate of 375–500 g product per hectare in 1000–2000 L of water per hectare with a maximum of 2 applications per season is accepted for suppression of fire blight disease on pears and apples. The first application should be made at 15–20% bloom followed by a second application at full bloom-petal fall. Ensure thorough coverage of blooms. Use higher rate (500 g product/ha) under high disease pressures.

Bloomtime Biological FD Biopesticide is compatible with streptomycin and should be used in an integrated fire blight suppression program with streptomycin. Copper-based formulations are incompatible with Bloomtime Biological FD Biopesticide.

7.5 Unsupported Uses

All proposed uses have been satisfactorily supported by the applicant.

8.0 Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, has granted conditional registration for the sale and use of Bloomtime Biological Technical Biopesticide and the end-use product, Bloomtime Biological FD Biopesticide, to suppress *E. amylovora* populations (fire blight) in apple and pear orchards. An evaluation of current scientific data from the registrant and published scientific reports has resulted in the determination that, under the approved conditions of use, the end-use product has value and does not present an unacceptable risk to human health or the environment.

Although the risks and value have been determined to be acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional confirmatory scientific information is being requested from the registrant as a result of this evaluation (see below) to ensure that *P. agglomerans* strain E325 can be properly distinguished from other strains of this bacterial species and to confirm the absence of human and animal pathogens from the final formulated product. The registrant will be asked to submit this information within the time frames indicated below.

Methods

- The following items are required to identify the MCPA and to confirm the absence of pathogens in the final formulated product:

    - An identification method to distinguish strain E325 from other naturally occurring strains of *P. agglomerans* is required. A genetic fingerprinting method has been developed for other *P. agglomerans* strains (McManus and Jones 1995), which could readily be adapted for this purpose. Submission of the method to the PMRA must be made no later than 1 December 2007.

    - To ensure that Bloomtime Biological FD Biopesticide does not contain any human and animal pathogens, the registrant will be required to include microbe-specific screening methods for *Salmonella*, *Shigella*, *Staphylococcus*, enteric bacteria, *Vibrio*, yeasts and moulds as well as *Pseudomonas aeruginosa* in the manufacturing process. Confirmatory data from five representative production batches will be required. Alternatively, if fewer than five batches of end-use product are manufactured in a 12-month period, then representative data from each batch produced in that timeframe will suffice. Submission of the method and representative data to the PMRA must be made no later than 1 December 2007.

At the time that a decision is required to either convert these conditional registrations to full registrations or to continue them as conditional registrations (with a new Section 12 Notice), a public consultation document on the proposed decision will be published.

List of Abbreviations

°C	degree(s) Celsius
a.i.	active ingredient
a_w	water activity (the amount of water available for hydration of materials)
CFU	colony forming units
DACO	data code
DNA	deoxyribonucleic acid
g	gram
GC-FAME	gas chromatography of fatty acid methyl ester
ha	hectare(s)
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LO	live organism
LPS	lipopolysaccharide
mL	millilitre(s)
MPCA	microbial pest control agent
MRL	maximum residue limit
MSHA	Mining Safety and Health Administration
N/A	not applicable
NAFTA	North American Free Trade Agreement
NIOSH	National Institute for Occupational Safety and Health
NYDA	nutrient-yeast-dextrose-agar
PMRA	Pest Management Regulatory Agency
TSMP	Toxic Substances Management Policy
TWG	Technical Working Group on Pesticides
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency

Appendix I Tables

Table 1 Toxicity and Infectivity of *Pantoea agglomerans* strain E325 and Its Associated End-Use Product (Bloomtime Biological FD Biopesticide)

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference
Acute Toxicity/Infectivity of Bloomtime Biological FD Biopesticide				
Acute oral toxicity and infectivity	Rat—Sprague Dawley 9/sex treated with MPCA, 1.05×10^8 CFU/animal	$LD_{50} > 1.05 \times 10^8$ CFU/animal	No clinical sign indicative of toxicity, no mortalities and no abnormalities on necropsy. The MCPA was detected in the cecum contents, brain, kidney, lungs, and spleen of treated rats on day 7 but was completely cleared from all organs and fluids by day 14. **NOT TOXIC, NOT INFECTIVE**	PMRA 46467802
Acute pulmonary toxicity and infectivity	Rat—Sprague Dawley 18/sex treated with MPCA, 1×10^8 CFU/animal	$LC_{50} > 1 \times 10^8$ CFU/animal	No clinical sign indicative of toxicity, no mortalities and no abnormalities on necropsy. The MCPA was detected in the kidneys of treated rats from day 3 to day 14 but completely cleared from all animals by day 21. **NOT TOXIC, NOT INFECTIVE**	PMRA 46467803
Acute dermal toxicity			Based on a comprehensive review of published literature, the data waiver request was found to be acceptable to fully assess the risks associated with the MCPA given the intended use pattern of the end-use product in lieu of toxicity testing. **WAIVER ACCEPTED**	PMRA 46467804[1]

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference
Intraperitoneal infectivity			Based on a comprehensive review of published literature, the data waiver request was found to be acceptable to fully assess the risks associated with the MCPA given the intended use pattern of the end-use product in lieu of toxicity testing. **WAIVER ACCEPTED**	PMRA 46467804
Primary eye irritation			Based on a comprehensive review of published literature, the data waiver request was found to be acceptable to fully assess the risks associated with the MCPA given the intended use pattern of the end-use product in lieu of toxicity testing. **WAIVER ACCEPTED**	PMRA 46467804
Dermal irritation			Based on a comprehensive review of published literature, the data waiver request was found to be acceptable to fully assess the risks associated with the MCPA given the intended use pattern of the end-use product in lieu of toxicity testing. **WAIVER ACCEPTED**	PMRA 46467804

[1] A single report addressing the data waiver request for the outstanding toxicity/pathogenicity studies was submitted.

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects, Comments	Reference
Terrestrial Organisms					
Vertebrates					
Birds	Oral Pulmonary Injection	No study was submitted. In a waiver request, the literature search showed no reports of adverse effects in wild bird populations despite the ubiquitous nature of the MCPA. Exposure to endotoxin LPS in the outer cell wall of the MCPA via the inhalation route may lead to an innate immune response to LPS or hypersensitivity reactions in wild birds. **WAIVER ACCEPTED**			PMRA 46467805[1]
Wild mammals	No study was submitted. In a waiver request, the literature search showed no reports of adverse effects in wild mammal populations despite the ubiquitous nature of the MCPA. Exposure to endotoxin LPS in the outer cell wall of the MPCA via the inhalation route may lead to an innate immune response to LPS or hypersensitivity reactions in wild mammals. Acute toxicity studies (oral, pulmonary) with rats treated with the end-use product showed complete clearance of the MPCA in mammals with no sign of toxicity/pathogenicity. **WAIVER ACCEPTED**				PMRA 46467805
Invertebrates					
Honeybees	Oral (dietary) Contact brood or hive	No study was submitted. In a waiver request, published studies reflecting wort-case exposure scenarios (direct dusting) indicated no adverse effects to honey bees when dusted with the MCPA or related strains. There have also been no incidents of negative impact to bee hives in the test plot orchards in limited field trials with the end-use product over the past three years. **WAIVER ACCEPTED**			PMRA 46467805
Other arthropods	Dietary	In a waiver request, published studies reflecting wort-case exposure scenarios (oral) indicated no adverse effects to lady bird beetles when fed *P. agglomerans*. Literature also indicated that the microbial pest control agent is isolated in association with insects in nature, often in a symbiotic relationship with the host insect. **WAIVER ACCEPTED**			PMRA 46467805
Earthworms	Acute	No study or waiver submitted. Test data are not required for Bloomtime Biological FD Biopesticide, as there are no reports of adverse effects noted in the published literature on *P. agglomerans* or its phylogenetically close relatives.			N/A

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects, Comments	Reference
Soil microbes	Acute	No study or waiver submitted. Test data are not required for Bloomtime Biological FD Biopesticide, as there are no reports of adverse effects noted in the published literature on *P. agglomerans* or its phylogenetically close relatives.			N/A
Vascular Plants					
Vascular plants	Acute	No study was submitted. In a waiver request, published literature indicated that *P. agglomerans* is ubiquitous in the environment and is only rarely attributed to plant disease. Reports in the literature did, however, reveal that the MCPA may cause slow-growing, smooth-surfaced circular galls on Douglas fir and other Western conifer seedlings. Efficacy trials conducted with the end-use product and related strains reported no development of phytotoxicity or phytopathogenicity to apple and pear trees. **WAIVER ACCEPTED**			PMRA 46467805
Aquatic Organisms					
Vertebrates					
Freshwater fish	Acute	No study was submitted. In a waiver request, literature indicated that, while *P. agglomerans* has been isolated from aquatic environments, there have been no reports of adverse effects on fish. **WAIVER ACCEPTED**			PMRA 46467805
Estuarine/ marine fish	Acute	No study was submitted. Estuarine and marine fish are not expected to be exposed to the MCPA.			N/A
Invertebrates					
Freshwater arthropods	Acute	No study was submitted. In a waiver request, literature indicated that while *P. agglomerans* has been isolated from aquatic environments, there have been no reports of adverse effects on aquatic arthropods. **WAIVER ACCEPTED**			PMRA 46467805
Estuarine/ marine arthropods	Acute	No study or waiver request was submitted. Estuarine and marine arthropods are not expected to be exposed to the MCPA.			N/A
Non-arthropod invertebrates	Acute	No study was submitted. In a waiver request, literature indicated that while *P. agglomerans* has been isolated from aquatic environments, there have been no reports of adverse effects on aquatic non-arthropod invertebrates. **WAIVER ACCEPTED**			PMRA 46467805

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects, Comments	Reference
Plants					
Algae Freshwater plants	Acute	No study was submitted. Data requirements were waived based on the terrestrial use pattern and the narrow range of crops and other terrestrial plants affected by wild strains of *P. agglomerans*. No reports of disease or other adverse effects in algae and in aquatic vascular plants caused by wild strains of this microorganism were found in the published literature. **WAIVER ACCEPTED**			PMRA 46467805

[1] A single report addressing the data waiver request for the outstanding environmental toxicity studies was submitted.

Table 3 Use Claims Proposed by Registrant With Original Application and Whether Acceptable or Unsupported

Label Claims Proposed by Registrant With Original Application	Accepted Label Claims	Unsupported Label Claims and Comments
Dilution Rates: Use the following dilution rates when applying Bloomtime Biological. Use the coverage characteristics of the application equipment and overall plant size to determine the proper volume of water needed. Spray solutions should be used within 24 hours of mixing. Application Rates: Apply Bloomtime Biological to flowering apple and pear trees at the following times and rates: 15–20% Bloom: Use 375 grams per hectare in enough water to thoroughly cover the open flowers. Full Bloom-Petal Fall: Use 375 grams per hectare in enough water to thoroughly cover the open flowers. Note: Suggested volume of spray is 500–1500 litres of water per hectare. At lower rates the spray must be adjusted so as to thoroughly cover the open blooms. Note: If more dilute sprays are necessary (2000–3000 litres per hectare) to get through flower coverage, then use 500 grams Bloomtime Biological per hectare. **Do not apply after fruit set. Do not apply through any type of irrigation system.**	Apply Bloomtime Biological FD Biopesticide at a rate of 375–500 g product per hectare in 1000–2000 L of water per hectare with a maximum of 2 applications per season for suppression of fire blight disease on pears and apples. The first application should be made at 15–20% bloom followed by a second application at full bloom-petal fall. Ensure thorough coverage of blooms. Use higher rate (500 g product/ha) at high disease pressure. Bloomtime Biological FD Biopesticide is compatible with streptomycin and should be used in an integrated fire blight suppression program with streptomycin. Copper-based formulations are incompatible with Bloomtime Biological FD Biopesticide. **Do not apply after fruit set.** Spray solutions should be used within 24 hours of mixing.	None
Compatibility: Do not mix with copper-based compounds. Bloomtime Biological is compatible with streptomycin. If mixing with other crop protection products, it is recommended that small compatibility tests be run prior to large-scale applications. Allow a minimum of seven days between application of Bloomtime Biological and oxytetracycline products. Copper-based formulations are incompatible with the performance of Bloomtime Biological.		

References

A. List of Studies / Information Submitted by Registrant

3.0 Impact on Human and Animal Health

PMRA 46467802 — Final Report: *Acute Oral Toxicity/Pathogenicity Study in Rats With a Microbial Pest Control Agent (MPCA)*. OPPTS No. 885.3050. 3 June 2004. Stillmeadow, Inc. Laboratory Study No. 7972-03. 21 pages. DACO 4.2.2

PMRA 46467803 — Final Report: *Acute Pulmonary Toxicity/Pathogenicity Study in Rats With a Microbial Pest Control Agent (MPCA)*. OPPTS No. 885.3150. 18 June 2003. Stillmeadow, Inc. Laboratory Study No. 7381-03. 19 pages. DACO 4.2.3

PMRA 46467804 — Bloomtime Biological FD Biopesticide Acute Toxicology Waiver Request. 6 February 2005. Northwest Agriculture Products. Project ID 05-PRA-108. DACO 4.3.3, 4.4, 4.5.2, and 4.9.

PMRA 46467806 — Bloomtime Biological FD Biopesticide Residue Waiver Request. 6 February 2005. Northwest Agriculture Products. Project ID 05-PRA-110. DACO 7.0

PMRA 1281836 — Wright, J. (jwrightch@comcast.net), 19 July 2006. Re: Bloomtime Biological FD Biopesticide (Submission No. 2005-2362, 2364): Request for clarification. E-mail to: B. Belliveau (Brian_Belliveau@hc-sc.gc.ca)

4.0 Impact on the Environment

PMRA 46467805 — Bloomtime Biological FD Biopesticide Non-Target Organisms and Environmental Expression Waiver Request. Northwest Agriculture Products. February 6, 2005. Project ID 05-PRA-109. DACO 9.2.1, 9.2.2, 9.3, 9.4.1, 9.5.1, 9.5.2, 9.6, 9.7, 9.8.1 and 9.8.2.

5.0 Value

PMRA 1073185 — Stockwell, V.O., and K.B. Johnson. 2003. *Chemical and Biological Control of Fire Blight of Apple*. DACO M10.2.2.

PMRA 1073192 Stockwell, V.O., K.B. Johnson, J.E. Loper and T. Temple. 2005. *Chemical and Biological Control of Fire Blight of Apple*. DACO M10.2.2.

B. Additional Information Considered

a) Published Information

1.0 The Active Ingredient, Its Properties and Uses

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2.0 Methods of Analysis

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5.0 Value

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ii) List of Unpublished Information Considered

3.0 Impact on Human and Animal Health

No unpublished information was considered in this evaluation.

4.0 Impact on the Environment

Vanneste, J.L. 1996. Honey Bees and Epiphytic Bacteria to Control Fire Blight, a Bacterial Disease of Apple and Pear. Unpublished.

5.0 Value

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